Cytotoxicity of microcystin-LR to primary cultures of channel catfish hepatocytes and to the channel catfish ovary cell line

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Introduction

We observed responses of channel catfish (*Ictalurus punctatus*) hepatocytes in primary culture to microcystin-LR (MC-LR) in order to develop biomarkers of effect for cyanobacterial hepatoxins. Livers in live animals and freshly isolated liver cells (hepatocytes) are highly sensitive to microcystins because the toxin is concentrated into hepatocytes by the bile acid transporter system. Work with rat hepatocytes has shown that microcystin MC-LR is taken into the cell cytoplasm where it inhibits protein phosphatases leading to hyperphosphorylated cytoskeleton proteins leading to collapse of the cell structure, followed later by cell death. Although fish have not appeared to be as sensitive to the microcystin class of hepatotoxins as are rats, the following data indicate that channel catfish hepatocytes share some MC-LR toxic mechanisms with rat hepatocytes.

Hypothesis

We propose that (1) primary cultures of channel catfish hepatocytes respond to the presence of MC-LR with the formation of abnormal morphology before loss of viability; and (2) there is a significantly greater sensitivity of hepatocytes to MC-LR in comparison with the channel catfish ovary (CCO) cell line, which would not be expected to possess the bile acid transporter.

Methods

Hepatocyte culture. Hepatocytes from channel catfish (75-120 g) maintained in aquaria at 20˚C were prepared essentially as described by Seddon and Prosser (1999, Comp Biochem Physiol 123A:9-15) except that cells were washed by centrifugation in phosphate buffered saline and resuspended in serum-free EMEM at a concentration of 1 million cells/mL after a selection for viable cells by centrifugation though 30% Percoll. The hepatocytes were incubated in Falcon Primaria 96-well plates, 100,000 cells per well in serum-free EMEM medium buffered with 25 mM HEPES in the dark at 20-22˚C. The medium was not changed throughout an experiment.

Channel catfish ovary (CCO) cell culture. CCO cells obtained from the American Type Culture Collection were seeded into 96-well plates in EMEM supplemented with 10% fetal calf serum at a confluency of 20% and exposed to doses of MC-LR for 96 hours at 30°C.

XTT cytotoxicity assay. XTT, a tetrazolium salt that is converted to its formazan derivative by metabolic reduction due to cellular dehydrogenases, was used to measure the viability of hepatocytes. The assay was performed according to the manufacturer's instructions except for the extended incubation times at 20-22˚C. Formazan was measured at 450 nm wavelength on a Synergy HT microplate reader. Hepatocytes cultured in 96-well plates were observed and photographed with the aid of a bright-field, inverted microscope.

Results

Viability measured as XTT reduction in primary cell cultures of channel catfish hepatocytes following exposure to microcystin for 5 days resulted in approximately 30% less XTT reduction for each 1 μg microcystin/mL compared with control cells without MC-LR. In addition, a dose response was evident for the concentrations of microcystin used in this experiment. After a 3-day exposure to 10 μg/mL microcystin, channel catfish hepatocytes in cell culture had rounded. This morphological change was observed before a change in XTT reduction was detectable. CCO cells required approximately 50 μg/mL MC-LR to attain a similar amount of XTT reduction as did 1 μg/mL in the hepatocyte culture.

Conclusion

Exposure of channel catfish hepatocytes in primary culture to microcystin-LR elicits a similar pattern of toxicity to that reported for rat hepatocytes, suggesting that there are common toxic mechanisms between the two systems upon which biomarkers of effect may be developed.